AMERICAN JOURNAL OF PHARMACY

AND THE SCIENCES SUPPORTING PUBLIC HEALTH

Since 1825

Vol. 111. JANUARY, 1939 No. 1

CONTENTS

Annual Subscription, $3.00 **Foreign Postage, 25 Cents Extra**

Single Numbers, 30 Cents. Back Numbers, 50 Cents

Entered as Second-Class Matter at the Post Office at Philadelphia, Pa., Under the Act of March 3, 1879

Acceptance for Mailing at Special Rate of Postage Provided for in Section 1103 Act of October 3, 1917 Authorized February 15, 1920

EDITORIAL

On these pages the editor offers his opinions, unshackled by advertising patrons and unrestrained by anything save a sense of the decent and the truthful. The editor, alone, is responsible for their type, their tone and their tenor.

THE DRUGSTORE OF THE FUTURE

ACCORDING to a New York daily paper the drugstore of the future, streamlined, highly departmentalized, and with an orderly display of merchandise, will be shown in the Hall of Pharmacy at the great New York World's Fair. A television telephone booth, animated displays, an endless parade of products in the show-window, and elimination of crowded counters are features of this store of the future which will occupy three thousand square feet of space. It is stated that plans for the store are based on a survey made by the Hall of Pharmacy workers of fifty drugstores of the New York metropolitan area, and from a questionnaire distributed among several lay persons. The latter study is alleged to have elicited from drugstore customers the reaction that the drugstore of the future should be more orderly, and so arranged that customers might have an opportunity for self-service in the purchase of certain products. One of the features is accordingly to be a large vending machine outside of the store, making possible the emergency purchase of many products when the pharmacy is closed! ! !

After reading the foregoing announcement and finding it difficult to believe that those responsible for the Hall of Pharmacy should have so utterly misjudged the future of the calling, the writer decided to submit the aforementioned article to his small group of graduate students in pharmacy. He wanted to get from them their reactions to the project, and he was rather pleased to find that they too observed that these fantastic prophets of the pharmacy of tomorrow had looked through the glass darkly, and had actually contemplated a plan, not for fostering and furthering the interests of real pharmacy but actually for destroying them. So significant were the comments of this little group of hopeful, eager and intelligent young people preparing for their life-work, that I am quoting herewith passages from several of them:

"The *bête noire* of the whole picture lies in the so-called vending machine. The fact that a person would be able to do his own dispens-

ing in cases of emergency places the pharmacy on the same basis as the patent medicine shop, the tavern and the pool hall. If the pharmacy of the future is to be turned into a 'penny arcade' with the prescription counter as a side line, how does the pharmacist expect to remain in public esteem?" (B. K.)

"The new Food and Drug Act will menace the standing of this so-called pharmacy of tomorrow with its devices for merchandising so-called emergency medicines. When formulas begin to appear on the labels of patent medicines, the pharmacist will find a new responsibility and vending machines are certainly not a part of the new picture." (W. K.)

"Whoever has planned this pharmacy of the future has given no consideration to the present trends in pharmacy, with the ever-increasing stringent drug laws which in turn add to the burden of responsibility laid upon the pharmacist. Certainly the vending machine for emergency medication has no room at all in such a picture. Summing up this proposed drugstore of the future as seen by New Yorkers, it is nothing but a glorification of a patent medicine shop, and not the vision of clear-thinking pharmacists who see in their work a real mission in the interest of public health." (A. M.)

"Those who organized this exhibit have not only neglected the important professional services of the pharmacist but have conceived the reprehensible idea of installing vending machines for medicines. This is by far the most childish notion ever involved in such a picture, and makes of the pharmacist an automaton, and of the profession a farce." (F. DeM.)

"What this display pictures to me is not the glorification of pharmacy but its degeneration. The emergency vending machine is an example of this degeneration. And pray what are emergency medications? Isn't it a fact that the physician is the logical person to prescribe for an emergency, and does it not require the intelligence of a well-trained pharmacist to supervise the dispensing of such emergency medications? It certainly seems to me that those responsible for this display are neither good historians nor safe prophets." (D. U.)

And I should like to have those "pharmaceutical Solons" responsible for this part of Gotham's great fair visit with my graduate class.

They might get some FINE ideas!

IVOR GRIFFITH.

ORIGINAL ARTICLES

A FURTHER CONTRIBUTION ON THE USE OF GELATIN IN EMULSION PRODUCTS

By L. F. Tice
Department of Pharmacy, Philadelphia College of Pharmacy and Science

THE use of a special gelatin as a stabilizing colloid for oil-in-water emulsion systems having a slightly acid reaction has already been described by the author. (1) (2) (3) This special gelatin was formerly distributed by the various members of the Edible Gelatin Manufacturers' Research Society under the name Pharmagel. This name has recently been changed to Pharmagel A to distinguish it from a second type of gelatin termed Pharmagel B having an entirely different field of application in emulsions. It is the purpose of this paper to explain the difference in these two products, their fields of application, and the manner of using them.

The method of using Pharmagel A consists in brief of using as the dispersion medium a gelatin solution adjusted to a pH of 3.2 with tartaric acid and containing alcohol or some other suitable preservative. To a given volume of this dispersion medium is added an equal volume of the oil to be emulsified, the mixture agitated thoroughly and then passed several times through a homogenizer to accomplish the necessary emulsification. Emulsions so prepared are extremely white and well dispersed, contain a minimal amount of added stabilizer and are unusually palatable.

Certain limitations in the use of this type of gelatin have already been mentioned. (3) The following is an explanation of these limitations and a description of the use of this second and quite different type of gelatin which may be employed in such instances without the difficulties experienced with the first type of gelatin.

Pharmagel A is derived from an acid treated precursor and by electrophoretic study (4) has been found to have an isoelectric zone in the region pH 8. In the region pH 3.2 it is both highly hydrated and positively charged thus providing the necessary requirements for its function as an emulsion stabilizer. This positive charge, however, is the source of considerable difficulty when one attempts to combine with it the various gums such as tragacanth, agar, acacia, etc., which are negatively charged colloids in aqueous dispersions.

The addition of a gelatin solution at pH 3.2 to a solution containing one of these gums results in the formation of a coacervate between the oppositely charged colloids. Such coacervates have been reported and described in excellent detail by De Jong and his co-workers (5) who have contributed much to the knowledge concerning these colloid systems. No attempt is to be made here to elaborate upon the theories relative to coacervate formation. Nevertheless, it should be emphasized that the protective action of a colloid is either partially or completely impaired in the presence of any second substance favoring coacervate formation. For this reason an emulsion made with Pharmagel A and containing agar or tragacanth does not provide a satisfactory product.

In certain cases the addition of such gums or other negatively charged substances to an emulsion is required, as for example, in the preparation of a mineral oil emulsion with agar or the preparation of a very dilute emulsion containing, say, 5 per cent. of oil wherein the use of a gum for its viscosity contributing properties is required to prevent an otherwise rapid rate of creaming. Then, too, in some instances an emulsion must be prepared containing either slightly alkaline ingredients or ingredients not compatible at acid pH values, and here again Pharmagel A cannot be successfully used inasmuch as it requires a definite acid pH value in order to develop its stabilizing action.

Pharmagel B on the other hand is prepared from an alkali treated precursor and has an isoelectric point of 4.7. At a pH of 3.2 it is quite ineffective as an emulsifying agent since at that pH it is not sufficiently removed from its isoelectric point to be adequately charged or hydrated, properties which are prerequisites for efficient emulsion stabilization. The use of a lower pH is inadvisable due to the excessively acid taste imparted to the product. Experiments have shown, however, that in the region pH 8 Pharmagel B acquires a sufficient degree of hydration as well as a strongly negative charge, thus providing for as effective a stabilization in that region as is accomplished by using Pharmagel A at pH 3.2. Since with this second type gelatin at pH 8 one has both a negative colloid and an alkaline reaction both of the major limitations of Pharmagel A as an emulsifying agent are met by its selection. Furthermore, it is conceivable that some may prefer to use Pharmagel B in products wherein either special gelatin might be used with satisfactory results. Pharmagel B

is also important since it is compatible with practically all of the commonly employed emulsifying agents.

In order to provide some practical information concerning the use of Pharmagel B as an emulsifying agent a description of the method of employing it in several types of emulsions follows:

To prepare a simple 50 per cent. oil emulsion one uses as the dispersion medium a 1 per cent. solution of this gelatin whose pH is adjusted preferably by the addition of from 0.5-1.0 per cent. sodium bicarbonate to the solution after the gelatin has been dissolved and the solution subsequently cooled below 50 degrees C. Sodium bicarbonate is selected because it adjusts the pH to approximately 8 even when used in excess. A slight excess over that amount required to produce a pH of 8 is sometimes desirable since emulsions of fixed oils not so buffered have been observed to slowly drop in pH until a lower and more favorable pH for gel formation was obtained which resulted in their thickening to the point of incipient gelation. As an alternative procedure the pH may be adjusted to 8 by the use of sodium hydroxide and then a small amount of sodium bicarbonate added to provide some reserve alkalinity. This method improves slightly the resulting flavor of the emulsion since less saline material is present. The aqueous dispersion medium must of course contain some added preservative such as 12 per cent. alcohol which is prescribed in the official emulsions of the United States Pharmacopœia as well as a flavor should the emulsion be intended for oral administration. Sodium benzoate as a preservative is ineffective at this pH although a concentration of 0.2 per cent. of sodium methyl parahydroxybenzoate was found effective over a test period of two months.

To the aqueous dispersion medium compounded as described above there is added an equal volume of the oil to be emulsified, the mixture is agitated vigorously to uniformly mix it and then passed through a homogenizer several times until thoroughly emulsified. For small scale production the hand homogenizer described in this journal (April 1935) provides a quite satisfactory means of mechanical treatment. The preparation of such an emulsion with gelatin provides a means of rendering oils and fats more compatible with many technical products such as cosmetic lotions, insecticides, etc., wherever oil is to be present as the dispersed phase. For example, if it is desired to add a small amount of oil for its emollient effect to a quince seed hand lotion the oil is made into an emulsion as directed above and then two parts of the finished emulsion added to the quince seed mucilage for

each part of oil desired in the finished product. For example, the following typical formula may be given:

Emulsion of Almond Oil 50 per cent.	10 parts
Methyl parahydroxybenzoate	0.1 parts
Perfume as desired	
Quince Seed Mucilage, to make	100 parts

The almond oil, added in this fashion, being already finely dispersed, will mix intimately with the quince seed mucilage giving a smooth homogeneous lotion. If on the other hand the almond oil is dispersed using only the quince seed mucilage a very poor dispersion results.

The preparation of flavor emulsions of volatile oils in place of the ordinary alcoholic solutions sold as extracts appears to be an excellent application for gelatin. The high cost of alcohol and the restriction against the use of the newer solvents for food products would seem to warrant the interest of the food industries in emulsified flavors. Since a flavor emulsion should contain from 2-5 per cent. of volatile oil depending upon the individual product, the finished emulsion must possess a viscosity high enough to prevent a rapid rate of creaming; the dilute emulsions being more prone to creaming than the more concentrated. Gelatin as a viscosity contributing substance in such products is not practical since when sufficient gelatin is in solution to provide the necessary viscosity the solution is subject to gelation on reduction in temperature. On the other hand, a viscosity contributing gum like tragacanth lacks sufficient stabilizing power to provide a fine dispersion of the oil. By combining these two substances one obtains both desired features and an excellent emulsion may be prepared. The method of procedure is as follows: A 33 1/3 per cent. emulsion of the flavoring oil, e. g., oil of lemon, is prepared using one part of the oil and two parts of a dispersion medium consisting of Pharmagel B, 1 per cent., and sodium bicarbonate 1 per cent. dissolved in water and homogenized. To fifteen parts of this freshly prepared emulsion is added eighty-five parts of a tragacanth solution made as follows:

Tragacanth	5-7.5 gm.
Preservative, such as alcohol	100 cc.
Water, to make	1000 cc.

and the product is thoroughly mixed. The finished emulsion will contain 5 per cent. oil in finely dispersed form and the creaming rate may be regulated by the amount of tragacanth employed. In preparing such a flavor emulsion the 33 1/3 per cent. emulsion of the volatile oil must be diluted, as soon as it is prepared, with the tragacanth sol; otherwise aldehydes present in some of the volatile oils will cause the gelatin to set to form an irreversible gel which cannot then be diluted.

Insofar as the preparation of emulsions of mineral oil and agar are concerned, gelatin may be used to good advantage. The negative Pharmagel B must be used since only it is compatible with the negatively charged colloid agar. It is now a generally accepted view that the presence of agar in a mineral oil emulsion is without therapeutic significance, but since its use is firmly established, many still desire agar as an integral part of any mineral oil emulsion. For those who wish to try gelatin in such a preparation the following formula with directions is presented:

Pharmagel B	5	gm.
Agar	2.5-5.0	gm.
Sodium bicarbonate	5	gm.
Vanillin	0.04	gm.
Syrup	100	cc.
Alcohol	60	cc.
Water, to make	500	cc.
Mineral oil	500	cc.
To make emulsion	1000	cc.

Dissolve the gelatin and agar in 100 cc. of water by the aid of heat, cool somewhat, then add the syrup, the vanillin dissolved in the alcohol, the sodium bicarbonate and finally enough water to make the solution measure 500 cc., add the mineral oil, mix intimately by vigorous agitation and then pass through the homogenizer several times until thoroughly emulsified. Place the emulsion in a closed container and allow to stand overnight whence it will be found to be set to a soft gel due to the agar. This is now passed in the cold through the homogenizer again, which will accomplish a mechanical

dispersion providing a fluid emulsion which will not again set to form a gel.

An emulsion of mineral oil with milk of magnesia is an example of an emulsion containing an alkaline ingredient which necessitates the use of Pharmagel B. Since the milk of magnesia by its presence produces the necessary pH adjustment no other alkali need be added to the gelatin solution. The preparation of such an emulsion is accomplished by using as the dispersion medium a 1 per cent. gelatin solution containing 5-10 per cent. of milk of magnesia U. S. P., alcohol, as a preservative, and flavor. This dispersion medium is intimately mixed with the mineral oil and then processed by homogenization in the manner previously described. The finished product is found to be somewhat more viscous than a plain mineral oil emulsion due to the fact that the magnesium hydroxide exists chiefly in the form of dispersed particles. It is also important that the product be shaken well before using to guarantee uniform dosage.

Pharmagel B may also be used as a synergist to aid in the stabilization of many emulsion products prepared with other emulsifying agents. In such cases, however, it should be borne in mind that only when such products are processed by homogenizing or passing through a colloid mill will the effectiveness of the gelatin be utilized.

In conclusion, the author wishes to emphasize the fact that the foregoing formulas are not offered as the best possible products obtainable but as examples of the application of gelatin in various emulsions. It is hoped that with the information presented those interested in the use of gelatin will be enabled to apply it in their products.

BIBLIOGRAPHY

1. *J. A. Ph. A., 24,* 1062 (1935).
2. *Drug and Cosmetic Ind.* (Aug., 1937).
3. *Amer. Prof. Pharm., 2* (April, 1936).
4. *Amer. J. Pharm., 109,* 29 (1937).
5. *Biochem. Ztschr.* (A series of papers beginning 1929).

CHEMICAL MICROSCOPY OF ESSENTIAL OILS

II. BITTER ALMOND OIL

By L. Wilson Greene
Aberdeen, Maryland

BITTER almond oil is obtained by distilling the ripe kernels of *Prunus amygdalus* Stokes, var. *amara* De Candolle (Fam. *Rosaceae*) and other kernels which contain amygdalin (1). Gildemeister and Hoffmann (2) state that very little of the commercial oil is produced from bitter almonds, but that the kernels of the apricot (*Prunus armeniaca* L.) are usually employed. Whether the oil is made from bitter almonds or from apricot kernels, it consists almost entirely of benzaldehyde which is present to the extent of 85 per cent. or more.

The fact that the oil contains such a large amount of aldehyde makes its identification relatively easy because there are several well-known reactions for characterizing such compounds. That bitter almond oil can be identified by the methods of chemical microscopy has already been reported by Rosenthaler (3), and the present study was conducted in an attempt to confirm that worker's observations and to investigate the microchemical behavior of the oil more fully.

The work reported here is a part of a general investigation of essential oil microscopy, an introduction to which has appeared elsewhere (4).

EXPERIMENTAL

Specimens Used

Specimen No. E-29. French, old sample containing a crystalline sediment of benzoic acid. Dodge & Olcott Co.

Specimen No. E-63. True, U. S. P., old sample containing a crystalling sediment of benzoic acid. Eimer and Amend label.

Specimen No. E-119. French, U. S. P., current production. Antoine Chiris Company.

Specimen No. E-120. French, free from hydrocyanic acid, current production. Antoine Chiris Company.

Specimen No. E-195. Natural oil manufactured in France, containing hydrocyanic acid. Current production. George Lueders & Co.

Specimen No. E-205. French, U. S. P. XI, from apricot kernels, current production. Dodge & Olcott Co.

Oxidation

A small drop of the specimen was transferred to a microscope slide and allowed to stand for a few minutes. The drop soon became a mass of white dendritic crystals of benzoic acid, formed by the atmospheric oxidation of the benzaldehyde in the oil. Another drop was covered with a glass slip and allowed to stand for some time at room temperature. Examination under the microscope after three hours showed dendritic crystals extending into the preparation from the periphery of the cover slip. These resulted, of course, from the oxidation of the oil at the edge of the slip. When conducting microchemical experiments with bitter almond oil, it is well to remember that crystals found around the edge of the cover slip may be those of benzoic acid and not a reaction product of the reagent being studied.

An alkaline permanganate solution was prepared as follows: potassium permanganate 7 g., sodium hydroxide 1.5 g. and water 90 cc. (5). A drop of this solution and a drop of specimen were mixed on a slide and covered with a glass slip. A brown amorphous precipitate of manganese dioxide formed at once and, after the preparation had stood for 15 minutes, anisotropic crystals as in Figure 1 were found at a magnification of 100X. A minute crystal of potassium permanganate placed in a drop of the specimen produced similar crystalline forms in about 10 minutes.

Reactions With Alkalies

When benzaldehyde is treated with a strong alkali it is oxidized to benzoic acid and simultaneously reduced to benzyl alcohol. This is known as Cannizzaro's reaction. Various alcoholic alkalies were caused to react with the bitter almond oil specimens and in every instance a crystalline product was formed, whether the specimen and reagent drops were intimately mixed or caused to flow together on the slide. Saturated solutions of potassium and sodium hydroxides in methyl, ethyl, n-propyl and n-butyl alcohols gave a variety of crystalline forms, none of which was sufficiently well-defined to be of much use for identification purposes. One reagent, potassium hydroxide in n-butyl alcohol, gave the most consistent results when

a drop of the solution was mixed with an equal quantity of specimen and covered. The crystals were anisotropic and best seen in polarized light at magnification of 100X (Figure 2). If the proportion of reagent to specimen was varied, however, a different crystal form resulted.

When concentrated ammonium hydroxide was used as the reagent, the crystals illustrated in Figure 3 were formed. The thin diamond-shaped plates are similar to those shown in Figure 1 and are undoubtedly benzoic acid. One specimen (E-195) did not give crystals with this reagent as consistently as did the other five specimens.

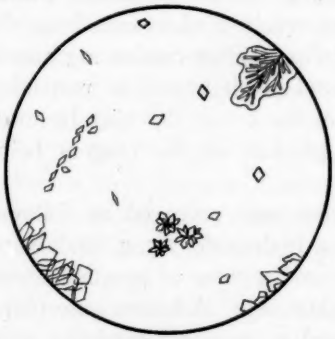

Figure 1
Bitter Almond Oil with Alkaline Potassium Permanganate

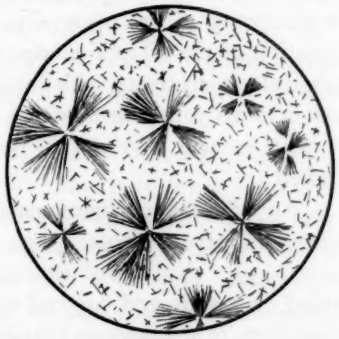

Figure 2
Bitter Almond Oil with Potassium Hydroxide in n-Butyl Alcohol

Reaction With Phenylhydrazine

Benzaldehyde reacts with phenylhydrazine to form a crystalline phenylhydrazone. Rosenthaler used this reagent and stated that needles and rods were formed with bitter almond oil.

A drop of specimen and a drop of phenylhydrazine (Eastman Kodak Co. product, m. p. 19-19.5 degrees) were mixed on a slide and covered. Yellow crystals formed immediately and when equal quantities of reactants were used the preparation solidified to a mass of interlacing needles. The best results were obtained when a drop of specimen about 2 mm. in diameter was mixed with a drop of reagent of about half that size. The crystals appeared as in Figure 4 when observed at 100X. They were anisotropic and showed strong interference colors when examined in polarized light.

When drops of specimen and reagent were caused to flow together, crystals of the same type were produced but they were longer and broader.

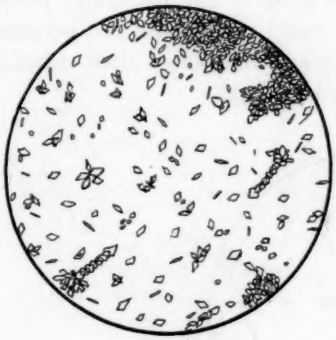

Figure 3
Bitter Almond Oil with
Ammonium Hydroxide

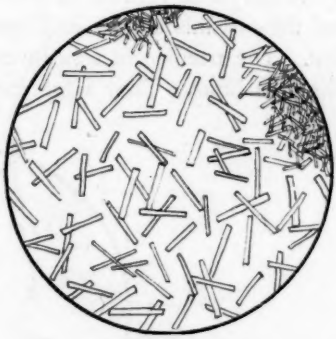

Figure 4
Bitter Almond Oil with
Phenylhydrazine

Reaction With p-Nitrophenylhydrazine

Rosenthaler recommended the following solution as a reagent for bitter almond oil: p-nitrophenylhydrazine 0.5 g., hydrochloric acid 1 cc., glacial acetic acid 7 cc. and water to make 50 cc. He stated that deep orange red needles were formed with this reagent.

The base not being available, a similar solution was prepared from p-nitrophenylhydrazine hydrochloride (Eastman Kodak Co. product), making the necessary adjustment in the above formula. A complete solution was not obtained, even with heat; the hydrochloride partially crystallizing out on cooling. The clear supernatent liquid was used and a drop of this, when mixed with a drop of the specimen, produced the orange red needles previously described. The crystals were very minute and could be resolved only with difficulty.

Reaction With Semicarbazide

This is another reagent proposed by Rosenthaler and was used in the form of a solution having the following composition: semicarbazide hydrochloride (Eastman Kodak Co. product), 5 g., potassium acetate 5 g. and water 15 cc. When drops of reagent and specimen were mixed, the preparation was filled with imperfect aniso-

tropic crystals of the semicarbazone. Better results were obtained when the drops were allowed to flow together by placing them close to each other on a slide and covering them with a slip. A white crystalline zone formed at once at the point where the drops joined and the crystals shown in Figure 5 formed throughout the preparation. The needle clusters formed first and the plates after standing about an hour. They were easily seen at 100X.

Figure 5
Bitter Almond Oil with
Semicarbazide

Reaction With Sodium Acid Sulfite

The formation of a crystalline addition product with sodium acid sulfite is a well-known reaction for aldehydes. Rosenthaler used a commercial solution of this compound (strength not stated) as a micro reagent for bitter almond oil.

The author found that a saturated aqueous solution of c. p. sodium acid sulfite was a very sensitive reagent for this oil. A 2 mm. drop of the specimen was caused to solidify to a white crystalline mass by stirring it with the end of a small glass rod moistened with the solution. When a large drop of specimen and a small drop of the saturated reagent solution were caused to run together by the technic described in the experiments with semicarbazide, anisotropic crystals like those shown in Figure 6 were formed. A 10 per cent. solution gave the best results when drops of specimen and reagent were intimately mixed and covered with a slip. In 5 minutes the crystals illustrated in Figure 7 had formed (100X). These were

anisotropic and showed interference colors in polarized light. No crystals were formed with a 1 per cent. solution.

Figure 6
Bitter Almond Oil with Saturated Sodium Acid Sulfite Solution

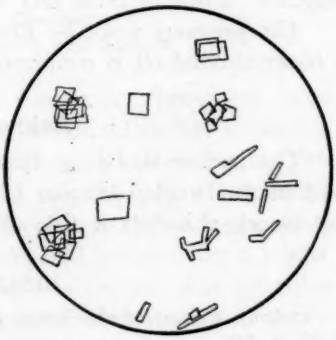

Figure 7
Bitter Almond Oil with 10% Sodium Acid Sulfite Solution

Discussion

The behavior of bitter almond oil on oxidation is apparently characteristic but the reactions with alkalies are not considered to be sufficiently reliable to be of much value.

The reactions with phenylhydrazine and sodium acid sulfite are believed to be specific since these reagents did not give similar crystalline products with about 100 other essential oils examined. Cassia oil, composed largely of cinnamic aldehyde, reacts with phenylhydrazine to produce small rod-shaped crystals but these have rounded ends. The greatest point of difference, however, is that the crystals of the phenylhydrazone from cassia oil do not show interference colors in polarized light while those from bitter almond oil exhibit strong colors. With sodium acid sulfite solution, cassia oil gives fine needles and spherulites which tend to dissolve in the preparation after standing for a few minutes. Semicarbazide may also give useful information in establishing the identity of bitter almond oil, but there is some evidence that fennel oil gives a somewhat similar reaction. The work with p-phenylhydrazine is considered to be inconclusive.

It is believed that cherry laurel oil (from *Prunus laurocerasus* L.), wild cherry bark oil (from *Prunus virginiana* Mill.) and the bark

oil from *Prunus sphaerocarpa* Ser., because of their benzaldehyde content, will give reactions similar to bitter almond oil with the above reagents. None of these oils was available for investigation.

The previous work by Rosenthaler on the chemical microscopy of bitter almond oil is confirmed.

Acknowledgment

The author wishes to thank the following firms for specimens used in this work: Antoine Chiris Company, Dodge & Olcott Co., and George Lueders & Co., all of New York City.

BIBLIOGRAPHY

1. Arny, *Principles of Pharmacy,* W. B. Saunders Co., New York (1920), 2d ed., p. 704.

2. Gildemeister and Hoffmann (translated by Kremers), *The Volatile Oils,* John Wiley & Sons, New York, Vol. II (1916), p. 577.

3. Rosenthaler, L., *Pharm. Acta Helv., 1,* 117-22 (1926); *Amer. J. Pharm., 101,* 191-6 (1929); *Perf. Essent. Oil Record, 21,* 277-86 (1930).

4. Greene, L. W., *Drug Cosmetic Ind., 43,* 156-8, 174 (1938).

5. Gildemeister and Hoffmann, *l. c.,* Vol. I, p. 299.

To Color Glycerin—For various purposes in plant and laboratory it is frequently desired to color glycerin. The following colors, in quantities varying from one to two ounces per gallon of glycerin, depending upon the depth desired, may be used:

Yellow—Auramine
Scarlet—Pylam Scarlet No. 1323
Green—Malachite Green
Blue—Methylene Blue
Orange—Chrysoidine
Violet—Methyl Violet
Black—Pylam Basic Black
Brown—Bismarck Brown

ORTHOTOLIDINE AND ORTHOTOLUIDINE TESTS FOR OCCULT BLOOD

By Louis Gershenfeld, P. D., B. Sc., Ph. M.
Department of Clinical Chemistry,
Philadelphia College of Pharmacy and Science

RUTTAN and Hardisty, of McGill University, presented more than a quarter of a century ago the orthotolidine test for occult blood (Jr. Can. Med. Assoc., Old Series, Vol. XLI, New Series, Vol. II, 999 (1912); Biochem. Bulletin, Vol. II, 225 (1913)). They stated that this test was more delicate, that the color produced in the reaction though it developed more slowly, persisted however for a longer period of time, and of special importance was the fact that the solution (4 per cent. orthotolidine in glacial acetic acid) remained unimpaired for from three to four weeks.

In recent literature, there has appeared statements calling for orthotoluidine. It happens that 4 per cent. solutions of both of these chemicals in glacial acetic acid will serve as tests for occult blood. However, the above workers are to be credited solely for the orthotolidine test and not for the orthotoluidine test. Who was responsible for the latter, or perhaps just when the typographical error first appeared may be difficult to determine. In all editions of "Approved Laboratory Technics," by Kolmer, Boerner and collaborators, it is listed as the orthotoluidine test. In the last issue of this volume on page 244 it is listed as the orthotoluidine test (Ruttan and Hardusty). The Hardusty is a typographical error for Hardisty.

Orthotolidine is a solid appearing as glistening pearly leaflets. Chemically it is 3,3'-dimethyl benzidine ($C_{14}H_{16}N_2$). Orthotoluidine is a colorless oily fluid and chemically there is present in its formula only one benzene ring (C_6H_4 (CH_3) NH_2). The latter is comparatively cheap in price costing about sixty cents a pound. The former is much more expensive, averaging about three to four cents a gram in 100 gram lots. It is of course apparent that orthotoluidine solutions are prepared with greater ease.

Orthotolidine solution added to aqueous dilutions of blood followed by the addition of peroxide of hydrogen is a more sensitive reagent than orthotoluidine solution added in the same manner. The latter when positive yields various intensities of violet rather than the different shades of blue given by orthotolidine.

However, the orthotoluidine reagent is of such sensitivity as to warrant its use for testing for occult blood in clinical laboratory procedures. Considering its cheapness and ease in preparation it may replace the orthotolidine reagent of Ruttan and Hardisty.

New Anti-Fogging Compositions

The efficiency of glycerin as a windshield wash, making for clearer vision and hence for safer driving, is a seasonal use for this versatile fluid with which many large transportation companies and individual truck and car owners are already familiar. In this connection, therefore, two new anti-fogging compounds utilizing the advantages of glycerine are of practical interest.

For those who prefer a liquid for use on windshields, windows and the like, there is a recent American patent (2,107,361) which consists of:

Glycerin	1 gal.
Camphor	4 oz.
Turpentine	½ pt.
Potassium oxalate	1 lb.
Oxalic acid	4 oz.

These ingredients are dissolved by heating together on a water bath. The mixture is applied by wiping the glass with a sponge dipped into the preparation.

In paste form is another somewhat similar formula for a windshield mist preventative for which users report good results. This composition calls for:

4 oz. . . Potassium oxalate	112 gm.
2 oz. . . Glycerin	60 ml.
1 gr. . . Camphor	0.1 gm.
1 oz. . . Turpentine	30 ml.

Heat on water bath.

The application of this product is reported to be beneficial both on the inside and on the outside of the windshield. The application to the outside of the windshield causes the rain to flow more evenly so that it does not reflect and refract street lights and the lights from other cars. On the inside, the application of the product prevents the accumulation of mist or fog and visibility is hence much clearer, thus making for easier and safer driving. The product is applied to a clean windshield and then rubbed well with a clean cloth.

THE TRAGIC NEGLECT OF SCIENTIFIC METHODS

By T. Swann Harding
Granite Gables, Falls Church, Va.

I

FOR eighteen years the writer of these lines was by profession a research worker in organic and biological chemistry. During that time he published his due quota of technical papers. For more than ten years thereafter, and until now, he has earned his living by acting as scientific editor of technical manuscripts in an institution where they appear at an approximate rate of one per working day, varying in length from half-dozen to over a thousand pages of typescript each. Certain quite definite conclusions emerge from this prolonged and somewhat devastating experience.

The most arresting of these is the fact that relatively so little attention is paid by research workers to the scientific methods they use. Scientific literature is cluttered endlessly with reports of research, new, often uncertain stabs of fact too hastily published. But rarely indeed does a scientist work conscientiously to test out methods, this probably being true in main because the chance of making a find of the type that leads readily to assured publication is less certain in this field, and a wide variety of factors conspire to tempt scientists into premature publication.

Yet, unless methods are sound, the results of work in one laboratory cannot accurately be compared with those from another, nor can any of the results even merit full reliance. A nutrition worker or a scientist active in medical research tries various food elements, vaccines, or infective viruses upon experimental animals. He then too often uncritically reports his results without sufficiently considering either the exact methods he used or the precise extent to which animal results apply to human beings.

He has not investigated the extent to which inbred experimental animals will tend to differ from one another even when kept under identical conditions. He may slur over individual idiosyncrasies in reaction to the same treatment. He may fail to control certain important conditions—humidity, temperature, light, the stock or control diet—or to consider how comparatively slight carelessness about such matters may greatly affect his results.

Let this not be regarded as an attempt to portray scientists as arrant fools. In part the difficulties of the scientist are inevitable,

ineradicable, and unavoidable. Like all of us he strives for a perfection he can never quite attain, but, unlike most of us, the very nature of his calling binds him to strive most self-critically. He must be ever alert to stamp out unnecessary and avoidable errors in his methods, to use his methods with the greatest care, and to make all necessary allowances for the somewhat artificial conditions under which he must experiment.

Consider so placid and profoundly lethargic an animal as the milk cow. Research studies are often made of her intake and output of such minerals as calcium and phosphorus in the effort to ascertain how much of these substances the lactating animal needs in her feed. But it is found that cows are easily disturbed by the conditions of such an experiment. They miss their usual exercise. They are excited by the presence of attendants and equipment. Their normal intake, use, and outgo of the minerals is violently disturbed by experimental conditions, just as the functions of the human stomach are violently disturbed by a gastric test meal supposed to tell the physician so much.

To what extent can a scientist expect that results obtained under such abnormal conditions will apply to cows or to human beings living what may be called more normal, if not more natural, lives? Who definitely knows? How can this gap of ignorance be bridged? The fundamental importance of research upon and standardization of methods is obvious. This is true throughout the field of science. It explains why chemists run "blank" determinations just to see how their equipment and reagents act under standard conditions.

Plant as well as animal specialists meet this problem too. Experimental plants are usually grown in greenhouse cultures, perhaps in water to which plant nutrients are added, not even in soil. It is then assumed the results obtained will apply to plants growing more naturally under field conditions. But the procedure of growing plants in synthetic culture media and noting the influence upon them of various chemical nutrients is highly artificial.

Such conditions are very different from those that obtain among plants growing in soil or in a field. Even pot growth in soil under greenhouse control is a method with severe limitations, and all such methods are most difficult properly to evaluate. How can the worker make just the right allowance for the inherent abnormalities of such procedures? He can check, of course, by growing similar plants in

the field and thus ascertaining differences caused by various soil properties and constituents.

One investigator called attention to these problems when studying the effect of wilt disease, caused by bacteria, on corn. He was seeking to test the natural resistance to wilt of certain sweet corn varieties. But if he grew his corn in the field he was at the mercy of nature. He must accept only the strains of the bacteria, virulent or non-virulent, that happened then to be present. He must face the fact that natural epidemics of wilt occur in widely varying degrees in different seasons, or in different corn fields in the same season.

The natural conditions favoring the most severe wilt often occur during a period in which even the most susceptible corn varieties are not infected and therefore appear as if resistant. Early strains that are susceptible may thus escape damage. Later, if the bacteria become more numerous or more virulent, or if other conditions so change as to promote infection, even quite resistant strains may appear as if susceptible. In any case many individuals, even though from quite susceptible strains, always escape infection in the field.

In part to avoid such difficulties the investigator may decide to grow his corn plants under highly artificial greenhouse conditions. Then he can definitely produce infection at their most susceptible stage of maturity; he can infect all of the plants and at the same time as well; he can standardize the type and amount of bacterial inoculum used, and he can so prepare it that all known types of the bacteria causing the disease are present. Then he can induce severe wilt infection even when conditions are most unfavorable for its natural occurrence.

So far so good—but how much have his final results to do with corn growing in the field under natural conditions and exposed to chance bacteria? What about the effects of factors such as climate, soil, altitude, fertilization, and other environmental conditions that so readily have an enormous effect upon results obtained? Even if he could control all these factors the investigator might still wonder just what relation existed between the results of such highly artificial techniques and what happens under natural field conditions.

II

By this time one may well ask whether a scientist can ever hope to prove anything at all by the use of artificial experimental methods.

The answer is yes, if he exercises care and works self-critically. For illustration we may take the disease called bean rust, caused by an organism with a name too long to seem quite proper. Laboratory conditions of experimentation cannot be made absolutely to duplicate field conditions, yet it is possible to discover in the laboratory why the rust occurs regularly in California, occasionally in Colorado, and never in Idaho.

For the use of carefully devised laboratory methods shows that no infection at all can take place if the relative atmospheric humidity is below 95 per cent., which is close to saturation. Infection occurs also only within a narrow temperature range of two or three degrees Centigrade. These temperature and humidity conditions must both obtain for nearly half a day for good infection to take place. Highly artificial laboratory experiments prove all this. The records of the weather bureau supply the remaining information needed.

These show that proper temperature and humidity requirements for bean rust infection occur daily in Southern California, where they are rendered just right for the required time every night by fogs coming in from the sea. In southern Idaho the temperature is often right but the humidity attains 95 per cent. for too short a time each night to permit infection. In northeastern Colorado, however, rain may occur at any time during the bean-growing season, and night temperatures are often right, so infection will occur every now and then.

Hence highly artificial experiments carried out under properly controlled conditions can teach the scientist much. Indeed he is compelled to resort to them because natural conditions are so ungovernable, and there are so many factors in raw nature that simply cannot be controlled. As one careful worker writes: "This attitude leads to a compromise somewhere in the planning of the experiment between the demands of good experimentation and the fear of the effects of limiting the 'natural', or the 'instinctive,' behavior of experimental animals"—and we may add plants.

Conventions are set up, but they must be followed rigidly. Perfection of and agreement upon methods, with rigid conformity thereto, are absolutely obligatory. Then other artificial aids must be invoked, such as statistics. But the tendency to resort to fancy statistical methods in the hope that they will atone for slovenly experimental work must be avoided.

Whole classes of objects must be judged from the behavior of random samples, limited in number, merely assumed representative of the whole, and always treated artificially. Physicists, chemists, and biologists can minutely examine only small samples of the total material at their disposal. The rest they must take on faith in the fiction of identity, though individuals in random samples are always variable —especially in biological and social fields. Indeed they are never absolutely uniform in physical science, for complete identity never occurs.

In the very first place the scientist in selecting a problem of sufficient importance to merit his attention has arbitrarily discarded any number of other potential problems. Once started upon his research he always tends to explain away error and to discard findings too lacking in conformity with the general trend of his results as a whole. Animals that die on experiment are usually not weighed and their weights added in for averaging; they are cast aside and their weights ignored.

Among the required broad, arbitrary assumptions there stands what is called "normal." But what is a normal? What is normal behavior? Certain insects sting trees and produce galls. These growths are abnormal for the tree, but not for the insect which requires them to survive. A cancer is an abnormal growth within the one afflicted, yet it can be said that a particular cancer grows normally. It is normal for certain fish to have two eyes when grown in sea water, but if grown in water containing "abnormal" salt mixtures they "normally" (shall we say?) have one eye.

What is a normal rat? No two rats are ever the same in total bodily composition, or in content of any chemical substance. Normal and abnormal are generally used to denote approach to or deviation from some standard that is usually itself unknown. At best "usual" or "average" is implied. Yet normal reactors are described as abnormal when in reality only certain causative factors have deviated from the average. On the other hand abnormal reactors are often dubbed normal because they failed to show normal responses to abnormal conditions!

It is usually said that a child is abnormal if it has undergone certain experiences that cause it to develop inhibitions or to behave differently from the average run of children. But if all "normal" children would develop similar inhibitions and behave similarly if sub-

jected to the same experiences, what then? This "abnormal" child must be "normal," in the light of experiences that were themselves abnormal! As a whole scientists have studied normals too little to be sure how they act, respond, grow, develop, and mature, nor have they investigated what they call "under normal conditions" sufficiently.

So far we have seen that scientific research is only possible with the aid of rigidly fixed and generally accepted assumptions. Here is a scientist who wishes to study the destruction of wood by an organism. It is necessary first to infect the wood under controlled conditions. Then the moisture and specific gravity of the wood must be determined initially. This is done by an arbitrary, conventional method. The stick of wood is first divided into four parts, the two end parts are discarded, the two middle parts are used.

Middle part *A* is dried to determine its moisture content; it is then soaked in paraffin and its specific gravity determined. But middle part *B* is reserved for the organism on the assumption that its moisture content and specific gravity would be identical with those of *A*—if determined. Such arbitrary assumptions must be made to get anywhere with the investigation at all. Yet the volumes of the actual sticks of wood inoculated are not determined. The volumes of precisely similar sticks are determined and are gratuitously assumed to be exactly the same as those of the inoculated sticks.

Such carefully agreed upon assumptions are needful even in rigid physical science. The very "potential energy" we learned about in school was an arbitrarily defined fiction. It was a purely passive entity needed to preserve the principle of the conservation of energy augustly described as one of nature's immutable enactments. But the potential energy of a system was so defined always as to permit the total energy of that system to be preserved intact.

If a system were found to be losing energy in some unaccountable manner, then it was assumed that mysterious energy radiations were taking place—or something of the sort—to preserve the fiction. Indeed the physicists of the nineteenth century essentially used the principle of the conservation of energy to define energy, for energy became that important something that was conserved.

This sort of thing is perfectly all right, just so long as it occurs consciously, so long as there is general agreement among scientists as to methods and assumptions, and so long as all workers rigidly

adhere to the same arbitrary conventions, methods and fictions. But it is as easy for a scientist to be tricked about the relationship between cause and effect as it is for a primitive, so carelessness must never invade his methods. Let us now consider this problem of correlating cause and effect.

III

A missionary tells a tale of a West African savage slave woman accused of killing the aged mother of a powerful chief. The woman was to be executed, but the missionary, being unable to believe that the old lady died as a result of necromancy, intervened, for he was sure that the "cause" of her death was quite "natural." To be sure the woman freely admitted her guilt, but the missionary begged permission to interview her alone. This was grudgingly granted, the chief being rather incensed that anyone should be reluctant to have a confessed murderess executed.

The slave woman herself insisted upon her guilt even when alone with the missionary, which baffled him. She claimed to have operated successfully using well-known malignant charms. She had collected crumbs of the old lady's food, strands of her hair, shreds of her clothing; she had mixed these with other substances and sung enchantments over them with drum and dance. She had tied the enchanted substances on a stick and secretly buried this beneath the old lady's door, fully expecting her to die. Naturally she did die within a month. Surely the cause of her death was quite obvious.

The missionary demurred. He could not believe that the woman had caused any such effect by that primitive method of hers. He was quite sure that a germ, or plain senility, had caused the old lady's death. But he found that he lacked any logical argument or appeal to common sense to convince the natives, so the slave woman was carried to sea in a small boat and there decapitated as was their custom. Indeed all science lacks a method of proving this primitive logic wrong once its premises are admitted.

By contrast consider a paper presented for publication by a scientist. It was to appear in a technical journal and to recount experiments in feeding dairy cattle different rations. The cattle showed "significant" differences in weight, depending upon the ration fed, and these differences were attributed to the rations as a cause. But the intestinal capacities of such ruminants are vast. Individuals may

vary in weight as much as fifty or sixty pounds merely because of differences in the quantity of intestinal waste matter present at different times. That true cause of the weight differences had been overlooked.

Such instances abound in science partly because scientists have been too hasty or too careless with their methods and partly because science unavoidably progresses step by step. Often assumptions have been made that such and such a food contained none of a specific food element, though later and more refined methods proved it did contain the element. It was once assumed that vitamin A could not be produced from carotin; but it was later found that the oil in which the carotin had been dissolved oxidized and destroyed all vitamin A present.

Certain investigators reported the curious finding that rats could not be rendered anemic by a particular iron-free diet. Later it was found that the rats chewed the zinc off the zinc-plated bars of their cages, arrived at the iron beneath, and so prevented their own anemia. Yet scientists usually consider it beneath their dignity to report upon such inconsequential details as the composition of cage bars and the gnawing habits of experimental animals.

Other investigators "proved" by experimental methods that young pigs could be prevented from becoming anemic by being fed iron citrate in certain manners. But close scrutiny of the work reveals that they had no specific blood count or hemoglobin test in mind as a standard for anemia, though it appeared that even their shoats judged to be anemic were not anemic.

A paper exists purporting to show that the vitamin content of spinach is correlated with variety, certain varieties containing more vitamins than others. Yet plant specialists say there are no spinach varieties; there is only spinach, disconcerting as that may be to the young. Furthermore the differences in vitamin content fell well within the limitations of experimental error in such work. Hence this elaborate paper with all its tables and graphs essentially correlated nothing with nothing.

A great deal of work was done upon what was regarded as vitamin A before someone refined the methods sufficiently to show that it consisted of at least two parts and vitamin D came into existence. A great deal of work was done on curing experimental animal rickets with foods supposed to contain vitamin D before refinement

of methods showed that sunlight, or light from a mercury vapor lamp, also cured rickets, and many successful results must be attributed to light, not food.

Obviously the scientist can easily be tricked. He can easily fail to find a true cause either because he uses a poor method or makes careless use of a good one. He may very easily attribute an effect to the wrong cause. Gradually his errors may crystallize into senseless routines and scientific superstitions arise—such as those that so long hallowed the inevitable preoperative purge in hospitals. This rite continued long, right in the face of evidence that it could not produce intestinal asepsis and that it debilitated the patient and diminished the chances of rapid recovery besides.

Senseless routines, scientific superstitions, and crass errors are common in medicine. For generations doctors used the therapy of depletion—starving, bleeding, and purging—right in the face of adverse facts. More recently medicine hit upon such routines as "removing foci of infection" in tonsils, teeth, gall bladder, or intestinal tract. Pathological entities were invented that probably had no existence in fact—prolapsis, stasis, intestinal autointoxication, neurasthenia.

Wise and critical physicians have called tonsillectomy "the American rite." Actually, of course, Hindu physicians removed tonsils centuries ago and Hippocrates advised that abscessed tonsils be drained. Celsus said pull the tonsils out with the fingers, though Galen described a snare for their amputation. As early as 1634 Peter Lowe published a book describing all varieties of tonsil removal. Today anything one desires to "prove" by the "literature" could be proved.

Physicians can fortify any belief they may have about tonsils because so much careless work has been printed. Yet the more careful studies have always shown that tonsil removal does not really accomplish a tenth of the good effects claimed for it. Follow-up studies made years after tonsils were removed demonstrate that the prophylaxis supposedly accomplished rarely occurred. Today there is a fortunately increasing tendency to question the value of this really rather dangerous operation as a protection against infections.

Another interesting group of fallacious medical treatment methods revolves around the high-blood-pressure and kidney-disease complex. Thousands of patients have been given all sorts of drugs to reduce

their blood pressures though careful tests show that no drug at all can be safely relied upon for this. Thousands have had their low-salt diets, disgustingly unpalatable, though careful tests show that such difficult, expensive and monotonous diets scarcely reduce the blood chlorides at all, as was supposed, nor the blood pressure.

Thousands of sufferers from hypertension have been told to restrict their meat consumption to "white," not "red" meats, or to eschew meats altogether, on the theory that their kidneys must be affected. But there is frequently no kidney involvement in high blood pressure; no cause-effect relationship has been proved. Moreover, even sufferers from kidney disease require a minimum of meat protein or else they tear down their own body tissues and metabolise them for maintenance. Finally, meat is meat, whether red or white; it is essentially the same chemically and nutritionally.

IV

Clinical and biological methods of testing drugs, serums, and hormone and vitamin preparations badly need reorganization and real standardization. As Professor Morris R. Cohen has shown, the reasoning at present used in testing a pneumonia remedy is, if made explicit, as follows: A cure for patents *X, Y* and *Z* is a cure for all pneumonia patients. Serum *A* is a cure for *X, Y* and *Z*. Therefore serum *A* is a cure for all pneumonia patients. Q. E. D. But one cannot be too careful in uncritically assuming that specific examples are typical of a wider class, and in permitting the phrase "uniformity of nature" to cover a multitude of errors in method or technique. "Refuge is thus found from the irksome task of taxonomy—the determination of just what characteristics do define classes uniform in other respects."

All clinical methods easily lead the more credulous worker into error. Drugs or serums are usually tested on all patients ill with a certain disease regardless of the natural virulence of the infection. The agent is often credited with saving lives really saved because the infection was light. Uusually all those who are sick are treated, because this seems "humane" though, in the long-time view, it would be more humane to treat one group and not to treat an exactly similar group in order permanently to evaluate the therapeutic agent.

There is no end of confusion in the standardization of glandular and vitamin products. Most of these preparations exist in the body

in very minute quantities. Many glandular products are sold as representing so many grains of the fresh or dried gland itself, whereas the gland content of the active principle is always variable. The standards and "units" so glibly discussed are often most unreliable.

There are many different kinds of vitamin units and of so-called "rat units." Vitamin standardization is more reliable now that several vitamins have been crystallized. But any unit devised in terms of an effect of a substance upon experimental animals becomes dubious when uncritically applied to human beings. Frequently the same effect may be produced in animals with several chemicals none or only one of which will cause the effect in human beings.

Often a drug is tested upon an animal in which it produces an entirely different physiological effect from that it is expected to produce in a human being. Sometimes one single element in a complex glandular compound is selected, and the test made for it alone, on the unwarranted assumption that the results will inerringly indicate the activity of a complex hormone itself.

Often an investigator confesses the unreliability of his method but leaps into print anyway. An example is the series of articles published to report the poisonous metal content of a wide variety of food substances, and hence their potential danger to human beings, determined by a method the authors admit to be shockingly inaccurate. The literature is full of such reports; either the methods were poor or they were improperly followed, yet this mass of print forms part of the record and must be winnowed and evaluated by later workers.

Take the long-accepted assumptions that the stomach secreted gastric juice only in response to food, that hunger was caused by waves of contractions in the stomach musculature, and that the "stomach-leaving time" of a food was a criterion of its digestability. A vast literature sprang from these assumptions before critical investigators in very recent times showed them entirely unjustifiable.

Tests of people enjoying good health disclosed that most of the symptoms and test responses used to diagnose particular gastric ailments were characteristic also of "normal stomachs," at least at certain times. Age factors, fatigue states, psychic and nervous conditions, sex and climate—even the time of the day, all tended to vary stomach functioning enormously. The amount of gastric secretion depended far more upon psychic factors than upon the kind and

amount of food eaten, while full stomachs showed as great and frequent "hunger" contractions as empty ones.

What is quite as important, the time foods remained in the stomach definitely was not, in any true sense, a relative measure of their digestibility. If this were true then milk must be regarded as less digestible than beef or salad. All the literature of food digestibility based upon the stomach-leaving time goes by the board.

As another example, consider the customary method of determining whether a food is nutritious by feeding it to test animals and attributing their weight gains thereto. Careful workers have shown that if groups of ten pigs be selected as nearly alike as it is possible to get them, and be housed, fed and treated in every way identically, individuals in the groups, and the different groups as compared with one another, will exhibit marked weight differences. These would have been regarded as significant and attributed to different rations, had such been used.

In the same way there are marked differences in the ash content of calcium and phosphorus and in the rapidity of healing rickets among rats kept on identical rachitogenic or rickets-healing diets. A great deal also depends upon whether the various groups of rats on quantitatively different diets number ten or a hundred. Much likewise depends upon whether the worker judges the extent of rickets by X-ray pictures, bone analyses, line tests, blood composition or growth.

The gain in weight of a test animal eating a test ration is called "growth" and is attributed to food elements in the test ration. But what is growth? It is just gain in weight? Does progress to maturity constitute growth? A child sick of a fever and in bed nevertheless elongates while it loses weight. Does it therefore grow? The tails and bones of rats fed deficient diets elongate while the animals lose weight. Have they grown? Scientists have failed precisely to define growth, and precise definition is of tremendous importance methodologically.

Assume for purposes of convenience that gain in weight is to be regarded as synonymous with growth. What about the chemical composition of the weight gain? One animal lays on fat, another bone, another water, another protein. The weight gain of one animal is more "dilute" than that of another. Some animals are more active than others; they burn up much food, whereas their mates use their rations economically for maintenance. The very metabolism of the animal may be speeded up or slowed down by the ration fed, or the

ration may depress their activity, or some element therein may serve as the precursor for some now unknown but most important dietary element animals need.

Assume next that animals lose weight on a diet deficient in vitamin A. Is that because vitamin A is lacking or because the animals got sick, lost their appetites, and could not then eat enough to obtain their vitamin A quota? Vitamin A is now added to the ration in abundance. The animals get well—or are they still suffering from deep-seated morbid changes caused by their former deficient diet? If used for another test later, as they usually are, can these animals be regarded as normal? Do animals lose appetite because they lack vitamins or do they refuse to eat because something else impaired appetite?

The responses of rats vary enormously, it has been found recently, with temperature and humidity; they must be kept in air-conditioned rooms to give reliable reactions. Under the best of conditions one rat may gain an ounce and a quarter in ten weeks on the same daily dosage of the same cod-liver oil that "causes" its litter mate to lose weight. Indeed there are recorded animals that lost weight on doses of the same oil fifteen times as large as those that produced gains in their mates. Moreover the margin of error in all such work may be reduced enormously simply by using more rats per test group.

V

If methods are imperfect, or if good methods are used with insufficient care, scientific progress ends and confusion begins. Fundamental research on methods is of basic importance and it is too much neglected. Truly comparable results cannot be secured unless methods are perfected and arbitrary assumptions are formally agreed upon. This holds true in all fields, and those scientists who realize the supreme importance of research on methods deserve commendation.

Some investigators recently interested themselves in the problem of how differently the same crop varieties grow in different soils and climates, at different altitudes, and with different fertilizers. There were marked differences in growth in a damp, coastal and a semi-arid, interior climate. Both yield and chemical composition of the grain were greatly affected. This held true even when the crops were cultured exactly alike, for the very manner of placing the fertilizer around a crop exerts a significant effect.

Oddly enough the differences persisted even when the coastal soil was removed to the semi-arid region and vice versa, the plants merely being grown in different climates and altitudes. The same plant variety responds also in varied ways to physical soil types and to invasion by organisms. It may react quite differently when every precaution is given to afford the same treatment. How much dependence can be placed upon the great masses of data upon crop growth as affected by different fertilizers when so many other obscure factors have been neglected?

When phytopathologists study the reactions of the innumerable varieties of wheat to the organisms that cause the destructive disease called rust, they soon become perplexed. The resistance of a particular variety to the same organism seems to vary. Often resort is had to the assumption that a new physiologic form of the organism has appeared, and there is learned talk of physiologic specialization. But a wheat may resist a certain form of rust in the fall and winter yet be somewhat susceptible to it in the late spring. Changes in temperature and humidity vary the type of infection caused.

If plants actually wilt with the rust the infected cells die off; then susceptible varieties may seem to be moderately or even highly resistant. It is very difficult to tell whether the more than fifty physiologic forms of the rust organism are themselves stable, or whether some truly differ from others. They are numbered and are usually distinguished from one another by the reactions to them of wheat varieties. But if thousands of varieties of wheat were used, and workers strained to find "new" physiologic forms of the rust organism, they could go far.

Where should they stop? Errors caused by environmental factors would increase with the fineness of the differentiations. Finer distinctions would require much more time, labor, space and expensive experimentation. Investigators have to call a halt somewhere, for there are numberless possibilities, and a structure or method could easily be elaborated that was too unwieldy to use. But it is very doubtful that sufficient critical attention has been devoted to this problem of the wheat varieties and the actually existing rust organisms. Other factors may now be producing effects attributed to new physiologic forms.

It is very fortunate that some scientists have, as we have seen, studied minutely the weight differences of certain animals when kept and fed identically, in order to determine what variations are to be

expected under controlled conditions. Others have studied cultures of various fungi and bacteria to determine the range of variation that must be expected in these lowly organisms under the best of control.

Others still have X-rayed the abdominal organs of healthy human beings only to find the "abnormalities" there to which a variety of symptoms are usually attributed. A few have painstakingly tested various analytical methods, often using complex apparatus, to determine the unavoidable margin of experimental error. Some have shown that the manner of placing fertilizers with relation to seeds and growing plants affects plant growth far more than very considerable variations in the composition of the fertilizing agent.

Broadly speaking, the scientist wanders into error not so much because he is stupid as because he is first and foremost a human being. Like all the rest of us human beings the scientist tends to be a hidebound conservative. He accepts, he does not rebel; he displays respect for authority in too many instances when healthy skepticism should make him boldly interrogate respectable beliefs generally held true without proof. He tends to accept at face value that which appears in print under the name of some notable authority. He tends not to question the axiomatic presuppositions in his field of science.

Like all of us the research worker too tends to repeat uncritically a procedure that appears to have succeeded before. A wide variety of factors literally drive the scientific worker on to print almost in spite of himself, and frequently when his findings are prematurely reported. Consequently he tends to deliberate and to question too little. Instead he rushes to perform some work by a more or less accredited method in order to get another paper into print. Yet, to do good work of lasting value, the research worker should question endlessly, especially those ordinary, commonplace, everyday, generally accepted axioms, definitions, assumptions, and methods he is so apt to use uncritically and with never an interrogation.

Much more attention to scientific methods is imperative. Research on methods should be held in far greater esteem. Sound, reliable, generally accepted methods must be developed before real progress becomes possible. Such methods are basic in science. They form the absolutely necessary firm foundation for all research worthy of the name and meriting publication. The popular application of scientific findings in practice may be anti-social or even dangerous unless research methods are unimpeachable. Without reliable methods science perishes.

ABSTRACTS FROM AND REVIEWS OF THE LITERATURE OF THE SCIENCES SUPPORTING PUBLIC HEALTH

Bacteriology . . . . **Louis Gershenfeld, B. Sc., Ph M.**
Biology . . . . . **Marin S. Dunn, Ph. D.**
Chemistry . . . . . **Arthur Osol, Ph. D.**
Pharmacy . . . . . **E. Fullerton Cook, Ph. M.**
and their assistants

New Plating Medium for Coliform Analysis—Citrate Ricinoleate Agar. M. L. Littman and C. N. Stark. *Jour. Amer. Water Works Assoc., 30,* 1808 (1938). A new method utilizing citrate ricinoleate agar for coliform analysis of potable and polluted water has been developed by the authors.

One liter of double strength agar is prepared by dissolving the following in 900 cc. of distilled water: 10 grams of bacto-peptone, 6 grams of C. P. anhydrous sodium citrate, 4 grams of C. P. sodium nitrate, 2 grams of sodium ricinoleate (Eastman-Kodak), 4 grams of C. P. lactose and 30 grams of shredded agar. The mixture is heated in an Arnold sterilizer to dissolve the agar. After filtering through cheese cloth, 12 grams of skim-milk powder (dissolved in 120 cc. of hot distilled water) is added, the mixture shaken, after which 100 mgms. of bromthymol blue (dissolved in 2.0 cc. N/10 sodium hydroxide and 40 cc. water) is added. The color at this point should be a deep green. Finally, 100 mgms. of neutral red (dissolved in 40 cc. distilled water) is added. The medium is placed in tubes, sterilized at 15 pounds pressure for 15 minutes, cooled, and stored in a refrigerator.

Procedure for coliform analysis: To each of five Petri dishes containing 10 cc. of water sample is added 10 cc. of the melted agar (50 degrees C.). After mixing the contents the plates are incubated at 37 degrees C. for 24 hours. At the end of this time the following counts are made: (1) The number of red colonies not exhibiting alkaline change—the "Escherichia" count; (2) The number of colonies remaining; (3) The number of proteolytic colonies (after flood-

ing plate with a protein precipitant, such as acidic 15 per cent. mercuric chloride, and allowing to react for 30 minutes. Proteolysis is indicated by a clear zone around the colony). The second count minus the third is the "Aerobacter" count.

The advantages of this method are: (1) a complete bacteriological examination of water can be made in 24 hours; (2) the coli index is obtained directly from the count. K. S.

High Pressure and Change of Color in Organic Solutions. *Science News, 89,* 12 (January 6, 1939). A report of the work of Drs. R. E. Gibson and O. H. Loeffler, of the Geophysical Laboratory of the Carnegie Institution of Washington, announces the discovery that when pressures of 1000 atmospheres are applied to a solution of nitrobenzene and aniline the orange color becomes more red as the pressure increases. Through this new discovery a strange link between a purely mechanical happening and one which is associated with atoms and molecules appears to be established.

Precisely what happens is a matter of speculation. It is suggested that increase of pressure increases the number of times per second that aniline molecules collide with nitro groups with a resultant increase in the number of times temporary formation of unstable compounds may occur. Perhaps it is these unstable compounds which give color to the solution. In any event, further research will be necessary before anything definite may be stated.

The new discovery suggests the possibility of utilizing color changes as a convenient and simple method of estimating pressure changes in high pressure apparatus or systems. A. O.

The Vacuum Drying of Extracts. H. Berry and E. M. Temple. (An abstract of paper read before British Pharmaceutical Conference.) *Austral. J. Pharm. 19,* 1049 (1938). The active constituent of krameria root is a water-soluble phlobotannin, krameriatannic acid, which decomposes readily into a phlobaphene, krameria red, which is present to some extent in the root and is insoluble in water. This is deposited from the extract on standing and the di-

rections for the preparation include both vacuum drying and concentration. Five batches were prepared in the laboratory and were found to contain from 1.8-8.4 per cent. of residue insoluble in cold water. The commercial samples were examined and found to range in color from light brown to very dark brown and the amount of water soluble residue varied from 9.6 to 52.6 per cent.

By experiment it was found that the factor responsible for such a high percentage of insoluble residue is undoubtedly that of overheating. The directions for making this product should state that the vacuum concentration and evaporation should be conducted at a temperature not exceeding 80 degrees C. The limit of water insoluble residue should not exceed 10 per cent.

In the case of extract of cascara sagrada as much as 30.8 per cent. of water insoluble residue was found in commercial extracts, and such insoluble portion was devoid of activity. The authors point out that in the final stages of vacuum evaporation when the extractive thickens and commences to dry the portion in contact with the pan will take the temperature of the steam irrespective of what reduction in pressure is employed. Therefore, if high pressure steam is used considerable overheating will result. This usually produces large variations in appearance, solubility, and probably in activity. A limit of 10 per cent. insoluble matter is recommended for dry extract of cascara sagrada. L. F. T.

The Preparation of Alkaline Buffered Solutions of Procaine Hydrochloride for Surgical Use. K. Bullock. An abstract of a paper read before British Pharmaceutical Conference. *Austral. J. Pharm. 19,* 1051 (1938). In 1910 von Gros described the increased activity of procane hydrochloride solutions when made alkaline and in 1913 von Ganza recommended the use of tablets containing sodium bicarbonate for the preparation of small quantities of solution immediately before use. The advantages claimed for alkaline solutions may be divided into three groups: (1) Acid solutions may cause pain at the time of injection; (2) Tissues recover better when treated with alkaline solutions; (3) The rate of onset of anesthesia is quicker and a smaller quantity of anesthetic will suffice. It has been shown that a 1 per cent. solution at pH 8.4 is 7.8 times as active as a solu-

tion of the same strength at pH 3.2. Procaine hydrochloride is stable in acid solution below pH 5.0 but it decomposes rapidly, especially if heated, in solutions of pH over 7.0. It has been shown that procaine-adrenalin phosphate buffered solutions reduced the unpleasant after-effects of spinal anesthesia. The problem of dispensing a sterile solution in which the procaine is undecomposed in rendered more difficult by the addition of adrenalin to the solution, as adrenalin is more unstable than procaine in alkaline solution. Three methods are available:

1. Procaine-adrenalin-bicarbonate or phosphate tablets may be used. If the tablets are sterile, and preserved and made up under aseptic conditions this would suffice; but the latter condition is a difficulty.

2. Separate solutions of procaine hydrochloride, buffering salts and adrenalin hydrochloride may be prepared and sterilized individually. These are then appropriately mixed prior to use.

3. The best method seems to be Fisher's dry ampul the salts in which are dissolved in sterile distilled water just before injection. These ampuls enclose a dry composite powder in a sterile condition. The preparation of such ampuls is described.

In addition to the above information the author discusses the stability of procaine solutions and the use of a buffer so selected that when the free procaine is absorbed into the lipoid matter of nerve tissue the hydrochloric acid which is split off will not result in a drop in pH below 7. L. F. T.

Polarimetric Determination of Calcium Gluconate. C. N. Tonesco and N. Stancin. *J. Pharm. Chim. 28,* 283 (1938), through *Analyst 63,* 902 (1938). The specific rotatory power of calcium gluconate is very low, making its determination in dilute solution almost impossible by the polarimetric method. The addition of various substances such as bismuth nitrate, uranyl acetate, tungstates and molybdates have been found to increase the rotation. Since ammonium molybdate produces the largest increase it has been made the basis of a method of determination. Ten cc. of a solution of calcium

gluconate are treated with 0.5 cc. of glacial acetic acid and 4.5 cc. of a saturated solution of ammnoium molybdate. The rotation of this solution is measured in a 2 dm. tube at 20 degrees C., the sodium D line being used. For concentrations less than 4 per cent. the rotation is strictly proportional to the concentration which can be calculated from the expression:

$$C = \frac{a \ 100}{222.67 \times l}$$

where C is the concentration in gm./100 cc. of the original calcium gluconate solution, the volume of acetic acid and molybdate reagent being ignored, and l is the length of the polarimeter tube in dm. The specific rotatory power of calcium gluconate under these conditions is 226.67 degrees. When the concentration of calcium gluconate exceeds 4 per cent. the solution must be diluted. The method is quite sensitive for the angle of rotation obtained under the conditions specified is comparatively large. Thus a 2 per cent. solution gives a rotation of + 8.98 degrees; without the molybdate it is only 0.36 degrees. L. F. T.

Nature of Vitamin A in Cod Liver Oil. A. O. Tischer. *J. Biol. Chem. 125,* 475 (1938). A concentrate of vitamin A derived by molecular distillation of Norwegian cod liver oil was allowed to stand at room temperature for 10 days with maleic anhydride in benzine solution. From the reaction mixture a compound $C_{44}H_{64}O_8$ was isolated having a melting point of 219-220 degrees C. This was the maleic anhydride adduct of vitamin A palmitate since the compound obtained in the same way from maleic anhydride and vitamin A alcohol esterified with palmityl chloride gave no depression of the melting point when mixed with it. Vitamin A palmitate is considered therefore as existing in cod liver oil. L. F. T.

Hydrogen Peroxide and Sodium Perborate: Their Comparative Oral Irritant Action. S. C. Miller, S. Sorrin, et al. *J. A. D.*

A. and D. Cos. 25, 1957 (1938). Considerable perplexity persists in the mind of the dentist concerning the use of hydrogen peroxide and sodium perborate in his practice. In one extreme is found the indiscriminate and unguided use of these agents by the patient recommended for the maintenance of oral health. In opposition to this view is that which holds these drugs to be indicated only in certain oral diseases and as to be prescribed for temporary use only as specific therapeutic agents. The basis of the latter view depends upon certain untoward effects which have been described as following the continued use of these materials in more or less concentrated solution.

The authors conducted a controlled clinical study of the oral use of sodium perborate and hydrogen peroxide with 181 subjects and found that, under the experimental conditions employed, 6.8 per cent. of those using hydrogen peroxide and 21.1 per cent. of those using sodium perborate developed hypertrophied (hyperkeratinized) filiform papillæ of the tongue. Other findings having a bearing on this subject are reported. The authors conclude by stating that the possible causation of oral pathosis by the use of these substances indicates the need for their use only under supervision as specific therapeutic agents with definite instructions to the patient as to the concentration to be used, frequency and method of use, and the need for rinsing the mouth with water after the solutions touch the oral tissues. Systemic effects are also possible from the excessive use of sodium perborate. L. F. T.

SOLID EXTRACTS

By Ivor Griffith, Ph. M., Sc. D.

Despite the form in which this information is presented it may be accepted as trustworthy and up-to-date. Original sources are not listed but they may be obtained upon request.

Farmer boys who remember with pain the pestiferous penance of picking feathers from the freshly killed makings of Sunday's chicken pie will be glad to learn that modern poultry preparation has been put on a machine basis.

Listen: Methods of poultry slaughtering which result in maximum bleeding and feather-muscle relaxation have been recently devised and this operation can now be done by machine with greater uniformity and less damage to the birds than by manual methods. The fowls are then transported by chain conveyors to tanks of water at 126-130 deg. F., in which they are held for 15 to 20 seconds and then roughly plucked. The 25 per cent. or so of feathers and hair remaining are dried, and the birds are plunged into molten wax mixtures. After the wax coating thus acquired has cooled, it is removed in large pieces that carry with them the remaining feathers. Although this method of feather removal has been experimented with for the past thirty years, the first successful commercial process was disclosed about 1931. The wax, of course, is reclaimed.

Did you ever know that glass is a heat trap and that its use in outdoor cold frames operates on that principle? Did you know that—whether a roof is dark or light in color has much to do with the temperature of a warehouse. Oil tanks that are painted white, or aluminum, stay relatively cool on hot days and warm on cool days. Glass is a peculiar substance in that it is transparent to high-temperature heat, as from the sun, yet is nearly opaque to low-temperature heat such as is radiated from ordinary objects. Window glass, therefore, acts as a heat-trap, allowing one to take the heat from the sun and not to lose it afterward, making possible warm conditions under a cold frame outdoors.

"Sugar is sweet
And so are You,"

is the essence of many cloying messages every St. Valentine's Day. But some sugars are as bitter as others are sweet. Sugar may be highly beneficial or poisonous, reactive in a chemical sense or conspicuously inert, extremely cheap or very expensive. There are literally hundreds of sugars, and many of them are available for varied technical uses.

So-called "rare" sugars are being produced from time to time from growing plants and by chemical synthesis, and several firms are active in their development and commercialization. Changes from rare to common are sometimes dramatically precipitous. Trehalose or mycose, a sugar that is found in fungi and in the manna of Persia, is possibly the most chemically stable of all known sugars, having the unique ability of resisting the combined action of alkalies and oxidizing agents.

The origin of words is so entrancing a study that we wonder why so few people take interest in it. Scientific words frequently have an odd origin. Take for instance the word TOXIN.

TOX, *which is the syllable whence comes such words as toxicology (the study of poisons), intoxication, toxemia, antitoxin, etc., is of Greek origin. The Greek neuter noun* "toxon" *means a* "bow" *and in the plural was used for the bow and arrow and even for arrows alone. Arrows tipped with poisons, such as snake venom, have been used in warfare since the dawn of history and the mutation of this Greek root as it came to us through the French, is thus readily accounted for. Indeed so recent an authority as the New English Dictionary defines the word Toxology—as the art of the bow and arrow.*

It may be of further interest to note that the Hebrews use the same word to describe both the bow of warfare and the rainbow. To them that phenomenon was God's weapon forever hung high on clouds, a symbol and an earnest of His promise, never again to punish with flood and hurricane His sinning children.

"And it shall come to pass when I bring a cloud over the earth, that the bow shall be seen in the cloud and I will remember my covenant which is between me and you and every living creature of all flesh; and the waters shall no more become a flood to destroy all flesh."

Poison laws, such as the Pennsylvania and other State Poison laws, which insist that pharmacists record the sale of toxic materials upon a special register are in some respects farcical. For instance, any dealer may sell nicotine sulfate (insecticide—Black Leaf 40)—to anyone, yet this material contains enough wickedness in one little vial to poison ten families. The same is true of paris green, lead arsenate, &c.

Poison laws should be made more consistent. By the way, to Scotland belongs the honor of introducing the first law of poisons. It was passed in 1450 in the reign of James II, whose Court apothecary had a booth in the bellhouse in Edinburgh, and enacted that "all persons are forbidden under pain of treason to bring home poison for any use by which any Christian man or woman can take harm."

So those were the good old days—were they? And yet one has only to cast his recollections back some thirty years ago to be reminded that there were some things commonly practiced in the drug business which were in no sense entitled to be called "good." Time was when soothing syrup, and cough "cure" contained enough morphine and cocaine to start out any number of addicts to their fatuous, useless ends. Babies were swindled to sleep with morphine lullabies. Tonics were prepared with wood alcohol whereupon people went blind, and doctors thought a new eye disease had come. Then, anyone could buy laudanum, like treacle, by the quart, and opium by the pound, like Cheshire cheese.

And those were the "good old days"! ! !

Since we have kept to a poisonous course, suppose we leave our subject with a query, wondering what it was that the learned judge in the following episode kept out of public record:

Dr. Christison, eminent toxicologist and professor of medical jurisprudence at the University of Edinburgh, was giving his expert evidence as to the recognition of a certain poison sought for after death, in a criminal case said to the Judge: "Your Honor, there is but one deadly agent of this kind which no chemist can ever detect in the human body after death, and the name of that poison is" . . . when he was sharply interrupted by the Judge. "Stop, stop, Dr. Christison. It is much better the public should never know it."